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REARING SELECTED WESTERN COTTON INSECTS IN THE LABORATORY

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ABSTRACT

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Laboratory cultures of the following five species of Lepidoptera are continuously maintained at the Cotton Insects Biological Control Laboratory, Tucson, Ariz., as hosts for parasites and predators, and as test insects for ecological and nutritional studies: Bollworm, *Heliothis zea* (Boddie); tobacco budworm, *Heliothis virescens* (F.); beet armyworm, *Spodoptera exigua* (Hübner), saltmarsh caterpillar, *Estigmene acrea* (Drury); and the pink bollworm, *Pectinophora gossypiella* (Saunders). Improvements in the handling of the insect diet and the above-mentioned insects are discussed.

KEYWORDS: insect rearing, bollworm, tobacco budworm, beet armyworm, saltmarsh caterpillar, pink bollworm

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REARING SELECTED WESTERN COTTON INSECTS IN THE LABORATORY

By Raymond Patana[1]

INTRODUCTION

Rearing insects in the laboratory is an ongoing process, and more efficient methods are continually being adopted. Sometimes, very minor changes in techniques can have dramatic results, both positive and negative. This paper is a revision of a previous paper (*6*)[2] and incorporates changes in methods and equipment adopted since 1969 at the Cotton Insects Biological Control Laboratory at Tucson, Ariz.

DIET

Listed below are the ingredients for one batch of diet:

Ground baby lima beans	10,080 g
Brewer's yeast	1,920 g
Methyl-*p*-hydroxy benzoate	200 g
Ascorbic acid	230 g
Sorbic acid	70 g
Aureomycin	35 g
Water total	51 liters
Formaldehyde	64 ml
Gelcarin	600 g

The dry ingredients, except for the Gelcarin, are mixed in a blender for about 6 minutes with 24.7 liters of water at 110° F, which brings the mixture to about 118°. The boiling Gelcarin mixture is then added to the other ingredients and mixed together for 1 min, which brings the temperature of the diet up to about 140°. The diet is then ready for dispensing.

The diet batch is essentially 16 times the previous 1-gal batch with some minor changes and additions. The lima beans are now ground in a hammer mill (C. S. Bell Co., Model No. 10). Grinding the beans produces an instant-type

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[2]Italic numbers in parentheses refer to Literature Cited, p. 8.

diet, which is consistently uniform. The diet is instant to the extent that the ground beans are ready to use as needed and prior soaking is eliminated. The ground beans also contribute to the uniformity of the diet in that variations of water-absorbing qualities of different beans are minimized. The beans are washed and dried prior to grinding to eliminate dirt clumps generally found in the beans. The washing consists of covering about 200 pounds of beans with water for about 5 min, draining, and then rerinsing. The beans are spread out on 1/8-inch mesh hardware cloth-bottomed trays, placed on racks, and moved into the drying room (7). The beans are ready for grinding the following day.

The Gelcarin is suspended into 24 liters of hot water in a steam-jacketed kettle by means of an air-powered stirrer (Model 4 AM Air Motor, Gast Mfg. Corp.). Before the stirrer was acquired, the Gelcarin was suspended in water in a blender with a 20-gal capacity (VCM-80E, Hobart Mfg. Co.). This operation required transferring hot water into the blender and mixing it with the Gelcarin, then returning the mixture to the steam kettle for heating. The use of the air-powered stirrer has reduced the amount of handling and, probably, reduced the amount of energy used in the process.

DIET PUMP

Previously, the diet was dispensed with air pressure using a modified pressure tank (5). At present, diet is dispensed with a pump (Teel Rotary Screw Pump Model P804, W. W. Grainger, Inc.), which is mounted above a 20-gal jacketed kettle, and both are mounted on a rolling rack (fig. 1). A 2-inch reinforced hose

Figure 1.--Technician in foreground is filling 9/16-oz cups; technician in background is filling plastic storage boxes. Diet pump is at the left.

is attached on the intake end of the pump. This hose is used to pump the diet from the mixer into the kettle. When the mixer is emptied, the intake hose is connected to the valve on the bottom of the kettle. This is done by inserting a 2- by 1-1/2-inch reinforced autombile radiator hose, which is attached with a hose clamp, over the valve opening, and is pushed into the intake hose. The valve is then opened, and the diet is recirculated through the pump.

On the discharge end of the pump, a 3/4-inch cross-tee is attached by means of a short 3/4-inch nipple. In the two side openings of the cross-tee, two 3/4-inch pipe-to-hose adapters are inserted.

For handling the diet, two 6-ft lengths of 3/4-inch reinforced heater hose with standard hose fittings attached are normally used. The number of hoses used for dispensing the diet varies; as many as five and as few as two have been used at one time. Opposite the pump side of the cross-tee, a 3/4-inch gate valve is attached between the cross-tee and a 3/4-inch bypass hose. This hose is 5 ft long and serves as a transfer hose for emptying the mixer and as a bypass when dispensing. The bypass is necessary since this is a positive displacement pump. This hose also serves to agitate the diet during dispensing. The gate valve is used to unblock the dispensing hoses, which sometimes become partially blocked between batches of diet. The valve can be closed down until the diet is forced through the dispensing hoses. When the hot diet has run through the hoses, normal dispensing can be resumed. To add multiple dispensing hoses, double shutoff "Y" hose connectors can be attached to the side adapters on the cross-tee and hoses are then attached to the adapters. To control diet flow, sweeper nozzles (Nelson N-404c) are attached on the ends of the dispensing hoses. The ends of these nozzles are made with threaded adapters to which sweeper nozzles (Nelson N-26B) are attached. The hose nozzle allows the operator to regulate the amount of diet going through the hoses, and the sweeper nozzle directs the hot diet into a solid stream. The 3/4-inch hoses are used for making diet flakes, pink bollworm layered diet, and for filling plastic storage boxes and 16-oz cups. For filling 9/16-oz and 1-oz cups, the same nozzle arrangement is used, but a 3/8-inch washing machine hose is substituted for the 3/4-inch hose. The smaller diameter hose reduces the flow of diet so that the smaller cups can be filled uniformly (fig. 1).

The general procedure for using the pump and holding kettle is as follows: (1) To keep the diet hot, the water jacket of the kettle is filled with hot water prior to use. (2) Hot water is run through the pump and hoses to warm them up. (3) The diet is mixed in the vertical-cutter-mixer. (4) The diet is pumped from the mixer to the preheated kettle, using the bypass hose. While this is taking place, the dispensing hoses can also be used. (5) When the mixer is emptied, the pump intake hose is connected to the bottom valve of the kettle. When the valve is opened, it allows the diet to be drawn through the bottom of the kettle, through the pump, out through the dispensing hoses, and back through the bypass hose to recirculate. By using the kettle as a holding tank, the mixer can be emptied, allowing another batch to be mixed while the diet is being dispensed.

PUPAL AND MOTH HANDLING

For all lepidopteran species, except the pink bollworm, moths are kept in 1-gal glass jars lined with plastic bags 3-1/2 by 5 by 13 inches. The plastic bag is blown up inside the jar to fit the extremities of the jar. Pupae are

surface-sterilized with a 0.03-percent sodium hypochlorite solution, rinsed with tapwater, and placed inside the bag. The number per jar varies with the species. One strip of paper toweling, 2 inches wide, is hung inside the bag, and the top of the jar is covered with a 5-inch square of paper toweling, which is held in place with a cutout lid.

The bags are dated and the moths are allowed to emerge at 75° to 80° F. The jars are checked daily. When the first moth emerges, a feeding vial, with the neck inserted through a hole in a 1-in^2 piece of Saran screen, is placed on top of the jar. This capless, 1/2-dram screwcap vial is filled with a 10-percent honey solution. A V-shaped slit is cut in the toweling cover of the jar, and the feeding vial is inverted over the opening.

After the mating and preoviposition periods, which vary with each species, the jars are checked for eggs. The toweling from the jars is collected daily after oviposition begins.

Previously, after oviposition began, moths were transferred to clean, plastic bag-lined jars daily. Later, this was changed to every other day. Now, they are no longer transferred. Whatever increase in production was gained by transferring the moths was more than offset by the cost of the labor involved. At present, moths are kept in the same bag-lined jar for approximately 4 or 5 days after oviposition begins and are then discarded. By gathering only the toweling (tops and strips), egg-gathering efficiency has been greatly improved. First, fewer bags are used; second, less time is spent in transferring the moths; and, third, there are fewer escapees. The toweling with the eggs is washed in a 0.03-percent sodium hypochlorite solution in a Buchner funnel and allowed to stand for approximately 5 minutes; then, the solution is drawn off with a vacuum. The eggs are then put into trays and placed in a ventilated cabinet to dry. When the toweling with eggs is dry, it is placed in a 1-gal plastic container (Lily 10MP) and covered, and the eggs are allowed to hatch.

Beet Armyworm

The larvae of the beet armyworm, *Spodoptera exigua* (Hübner), hatch in approximately 72 hours at 84° F. To maintain the parent culture, approximately 50 larvae are placed on diet in a 16-oz cup filled one-third full. The cups are then closed with untreated paper lids. The number of cups implanted depends on levels of production. Eight 16-oz cups per day produce sufficient moths to provide in excess of 25,000 hatching larvae per day (*2*). The larvae pupate in 10 to 12 days at 84°. The pupae are removed from the frass and diet by washing, and can be separated by skimming them off the surface with a screen sieve. They are then surface-sterilized as described for the eggs, but without use of vacuum.

After pupae are washed, they are placed in plastic-lined jars (as described previously). Formerly, about 25 pupae were placed in each jar, but it was later found that up to 75 pupae per jar resulted in the eggs being more concentrated on the strips, thereby reducing the number of jars used. Beet armyworm larvae used as hosts for parasite and predator studies (*1*) were reared in plastic storage boxes (3-1/2 by 10 by 13-1/2 inches). The hot diet is poured in a thin layer around the sides, and to a depth of approximately 0.25 inch on the bottoms of the boxes. From 400 to 600 newly hatched larvae are implanted per box, and a three-ply wiping tissue is placed over the box and covered with the lid. The

tissue seals the space between the box and lid and absorbs excess moisture that otherwise would condense, forming wet spots. When held for 7 days at 86° F, the larvae are of suitable size for parasitization or other uses (*3*).

Tobacco Budworm and Bollworm

The larvae of both tobacco budworm, *Heliothis virescens* (F.), and bollworm, *H. zea* (Boddie), hatch in approximately 72 hours at 84° F. Patana (*8*) described rearing tobacco budworms using dry diet flakes. This method is now also used for rearing bollworms. Flakes are placed in plastic storage boxes containing cooled diet, 16 to 18 hours prior to use, to a depth of approximately 0.75 inch. The boxes are kept overnight at room temperature. This allows the flakes to absorb any excess surface moisture from the diet. For maintaining parent cultures of each species, 75 newly hatched larvae are implanted per box. This will yield an average of 55 pupae per box. Pupae are removed by washing after 14 to 16 days. A visual inspection of the bottoms of the boxes can be made to determine if the larvae have pupated. The pupae are washed from the boxes with a kitchen spray hose and collected in a screen basket. As previously described, pupae are surface-sterilized and placed in plastic bag-lined jars, approximately 35 pupae per jar. The jars are held at 78° to 80°. When moths first emerge, they are fed a 10-percent honey solution, and the jars are inspected daily for eggs.

When earlier instar larvae are needed for predator or parasite studies, 300 to 400 larvae per box are implanted without flakes to facilitate finding these smaller larvae, which are difficult to remove from the flakes. After the larvae have reached third or fourth instar, flakes can then be added to prevent cannibalism. The dried diet flakes are also used to isolate parasitized larvae for parasite rearing (*4*).

Saltmarsh Caterpillar

The larvae of the saltmarsh caterpillar, *Estigmene acrea* (Drury), hatch in approximately 96 hours at 84° F. The larvae are infested by two different methods, depending on production levels. First, 25 larvae can be infested in 16-oz diet cups, or, second, 150 larvae can be placed on diet in plastic storage boxes. After 14 days, the 50 larvae are transferred to 6-1/2 by 10-1/2 by 14-1/2-inch plastic storage boxes containing two 2-in^2 blocks of diet in two 5-in^2 fiber trays. The fiber trays can be changed, fecal matter can be removed, and more diet can be added if needed. The use of the fiber trays was adopted after it was found that if the fecal matter was allowed to accumulate it led to mold development. The bottom of this plastic storage box has been cut out and replaced with 1/4-inch hardware cloth. The screened box is placed into a whole plastic storage box of the same size. This allows about a 2-inch space between the two boxes. Into this space, shredded paper is placed for a pupation site. The upper box is covered with a lid containing a 5- by 8-inch opening covered with window screen. The screened top allows for an air exchange through the entire unit, thereby eliminating excessive moisture buildup. For maintaining the parent culture, approximately 25 14-day-old larvae are placed in the unit every other day.

During 1975 and 1976, this same method was used to hold saltmarsh caterpillar larvae parasitized by *Exorista mella* (Walker). During this 1-year period, more than 10,000 flies were produced for release in trials to control the range caterpillar, *Hemileuca oliviae* Cockerell (unpublished data). For maintaining the laboratory culture, 25 to 30 surface-sterilized pupae are placed in each jar.

Pink Bollworm

Pink bollworm, *Pectinophora gossypiella* (Saunders), eggs are put into layered diet cartons after 72 hours at 84° F and hatch as described by Patana (7). After hatching, larvae burrow in between the alternate layers of diet and waxed paper, which offer the larvae isolation and reduce cannibalism.

The layered diet is prepared by spreading hot diet on sheets of waxed paper 18 by 66 inches. It is allowed to cool and is then rolled into a compact roll 6.25 inches in diameter and 18 inches in length. The roll is then cut into slices 1.5 inches thick. The slices are partially dried overnight. In the morning, they are put into 1-gal cartons, three slices per carton. The remaining space in the carton is filled with a piece of Hexcel (fiberglass honeycomb material), 6 by 5-1/4 by 2 inches with 3/4-inch cells.

The larvae begin to chew their way out of these cartons after 14 days and are captured in pans of cornmeal. The larvae in the pans of cornmeal are changed daily and are allowed to pupate in the pans for 5 days. After 6 days, the excess cornmeal is screened off leaving only the pink bollworm cocoons with bits of cornmeal attached. The next day, the cocoons are washed in a 0.1-percent solution of sodium hypochlorite to dissolve the webbing from the pupae, which are then rinsed with tapwater and dried. After drying, pupae are measured (4 ml = 100 pupae) and placed in a cage 10-1/2 by 14-1/2 by 6-1/2 inches, 3,000 per cage.

The moth cages are held at 78° F. At this temperature, the moths begin to emerge after 2 days. After emergence, moths are fed daily by moistening a 2- by 3-inch cotton pad with a 10-percent sugar solution. An 8- by 10-inch plastic screen is placed on top of the cage screen, and on this is placed a 4- by 5-inch piece of mimeograph paper weighted with plastic to hold it flat against the cage. The mimeograph paper is checked daily for eggs, and when oviposition begins, an 8- by 10-1/2-inch sheet is substituted. This sheet is held down with six plastic weights. The mimeograph sheets with the eggs are changed daily.

The cage bottom is cut and replaced with 8- by 10-inch aluminum window screen, which is attached with hot glue. The lid also has a 5- by 6-inch section, which is cut out and replaced with screen. After the pupae are placed in the cage, the lid is taped on, and the cage is inverted and placed on a 30- by 10-1/2-inch pan, which is on a holding rack. The length of the pan allows three cages to be placed on it side by side. The space inside the pan under the cages serves as a collection area for moth scales that sift down through the screened portions of the cage lid. One of the cages can be removed, and the scales can be vacuumed out of the pan. By using this method, a large portion of the scales fall out of the cage into the pan where they are trapped so that they are not released into the room.

The 8- by 10-1/2-inch egg sheets are cut into quarters. The eggs are surface-sterilized with a 0.02-percent formaldehyde solution and rinsed with tapwater. They are allowed to dry and are then held at 84° F.

DISCUSSION

The laboratory cultures of beet armyworm, tobacco budworm, bollworm, and pink bollworm have been maintained in the laboratory for over 12 years without outside introduction.[3] This would indicate that these cultures are well adapted to laboratory conditions. In this time period, there has been no noticeable change in the number of eggs produced, percentage of egg hatch, number or size of pupae produced, or percentage of moth emergence due to inbreeding. Since these cultures are used mainly as hosts for parasites and predators, and are not in competition with wild-type species, no apparent culture deterioration has been noted.

The daily implanting of larvae--7 days a week--seems to be an important factor in maintaining these cultures for such a long period. This provides a continuous supply of pupae, which are put in jars or cages each day.

In rearing the tobacco budworm and bollworm with dry diet flakes, there has probably been some selection for less aggressive larval strains. When the diet flakes are left out of the boxes, very few larvae survive to pupation (fewer than 10 out of 75). When the flakes are present, it is thought that a certain amount of competition between the larvae tends to eliminate weak or injured individuals. This is possible since there is no actual restraining barrier formed by the flakes as there would be in individual cups, and the larvae are able to search out particular areas within the container suitable to their preferences.

In the various spaces for holding developing larvae, temperature and day length are controlled, but no attempt is made at humidity control. It is felt that by keeping the diet for newly hatched larvae at what is considered to be at the optimum moisture level (for whatever type container is being used), the humidity can be kept at an optimum level throughout the developmental period. Actually, what we try to achieve is a controlled drying affect. As the larvae develop, there is some moisture loss from the container, but not so great a loss that the diet dries out. If this moisture loss can be kept at a desirable level, then there is no great moisture buildup during pupation, and the chance of mold development is lessened. This controlled drying is probably better suited to a drier climate such as that of the Southwest. To achieve this controlled drying, several different methods are used with the different-type rearing containers. One thing that would apply to all the rearing containers would be to have the correct amount of diet per container. Other methods include partial drying of the pink bollworm layered diet before use and using untreated paper lids for 8- and 16-oz containers, triple thickness tissues under the lids of plastic storage boxes, diet flakes, and partially screened lids in the case of excessive moisture buildup.

The methods described here have been successful at this laboratory for both small- and large-scale rearing programs and could be adapted for use with other insect species.

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[3]The saltmarsh caterpillar culture was kept for about 8 years without outside introduction but was discontinued for about 2 years, and a new culture was started in 1974.

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